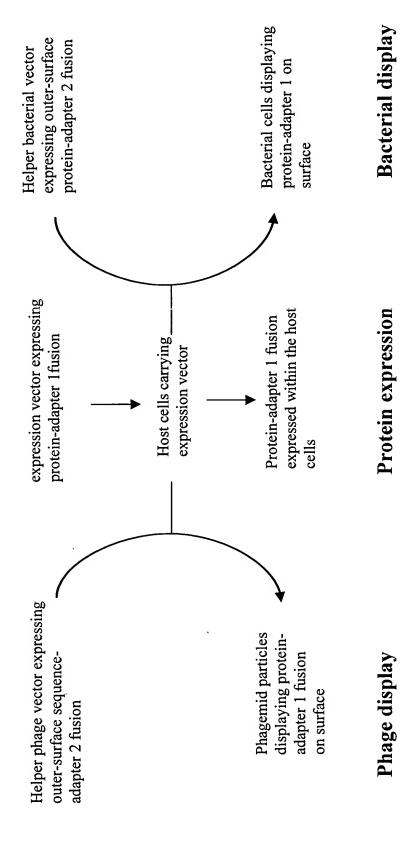
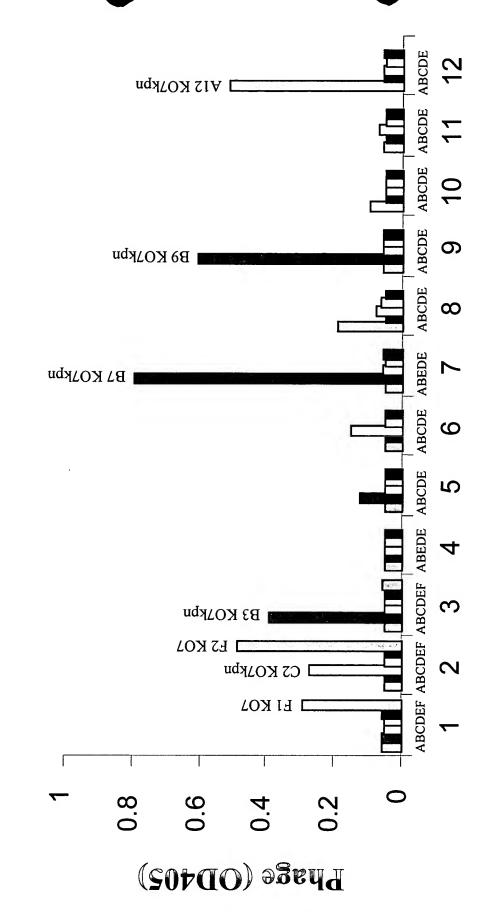
Adapter-directed display systems

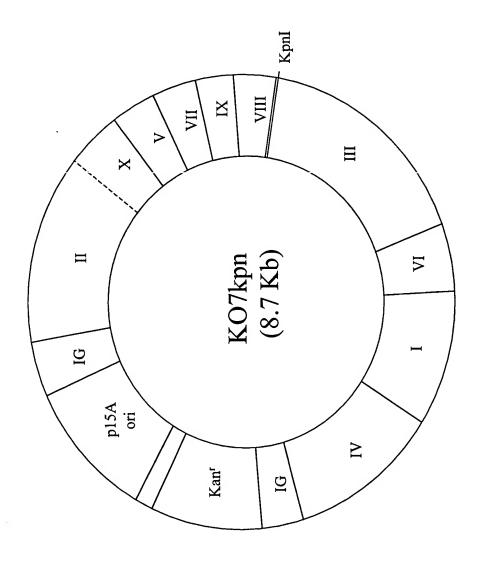


KO7kpn phage Screening by ELISA



H.

KO7kpn helper phage Vector



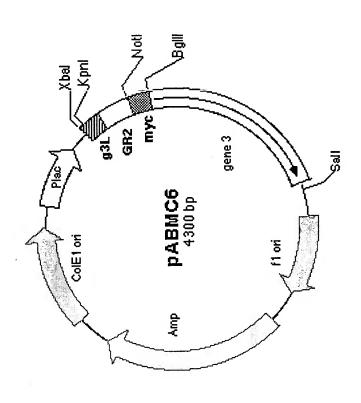
Gene III leader sequence in KO7 helper phage

GTG AAA AAA TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC TCC GCT V K K L L F A I P L V V P F Y S H S A

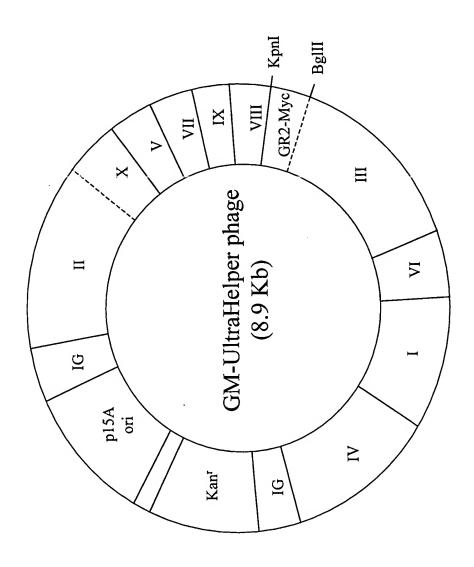
Gene III leader sequence in KO7kpn helper phage

GTG AAA AAA TTA TTC GCA ATT CCT TTA GT<u>G GTA CC</u>T TTC TAT TCT CAC TCC GCT V K K L L F A I P L V V P F Y S H S A

Map of phagemid vector pABMC6



Helper phage with engineered gene III fused to adaptor 2



GR2-Myc domain coding sequence in GM-UltraHelper phage genome

KpnI Gene III leader

---TTAGT<u>GGTACC</u>TTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGA 臼 Ø n L Ы ഷ ß ۲ ഗ Ħ L V V P F Y

GR2

NotI

ATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC GCGGCCGCA A A A U Ö Ö > û o Ы œ Σ H > 团 臼 Н Ω × Γ D

GAACAAAAACTCATCTCAGAAGAAGGATCTG AGATCTGGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA---ഗ Gene III T V ש ט м 8 BgIII ᆸ Д Myc-tag Q. K L I

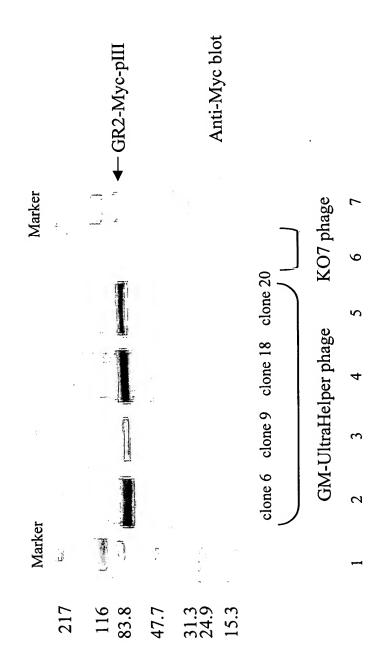
Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

GR2 domain TSRLEGLQSENHRLRMKITELDKDLE

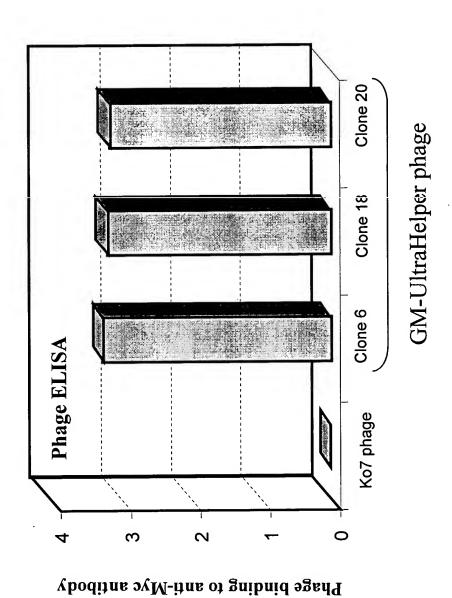
E C

TMQLQDVGGCAAAEQKLISEEDLRSGGG Myc-tag

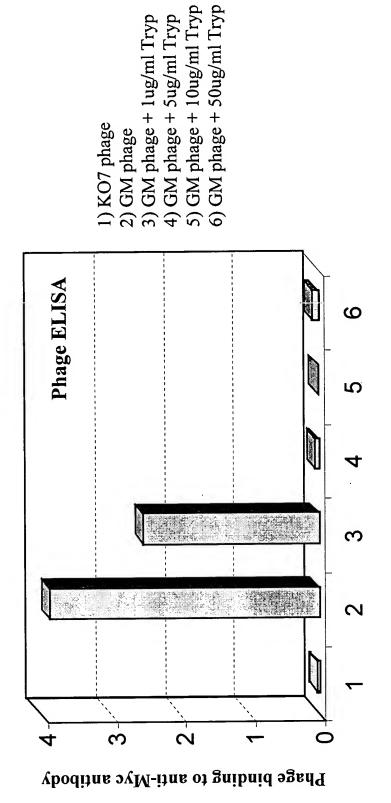
GR2-Myc-pIII fusions assembly into GM phage particles



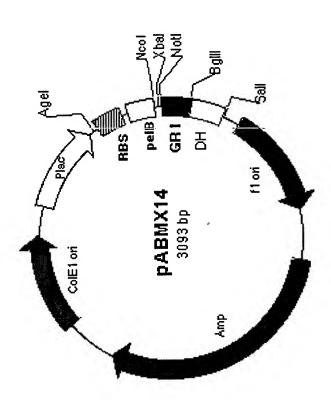
Detection of GR2-Myc domain on GM-UltraHelper phage



Cleavage of GR2-Myc domains on GM phages by trypsin



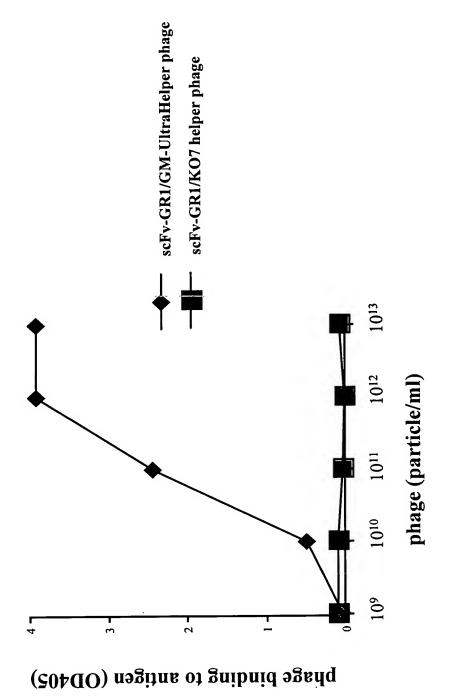
Phagemid vector for protein-GR1 expression



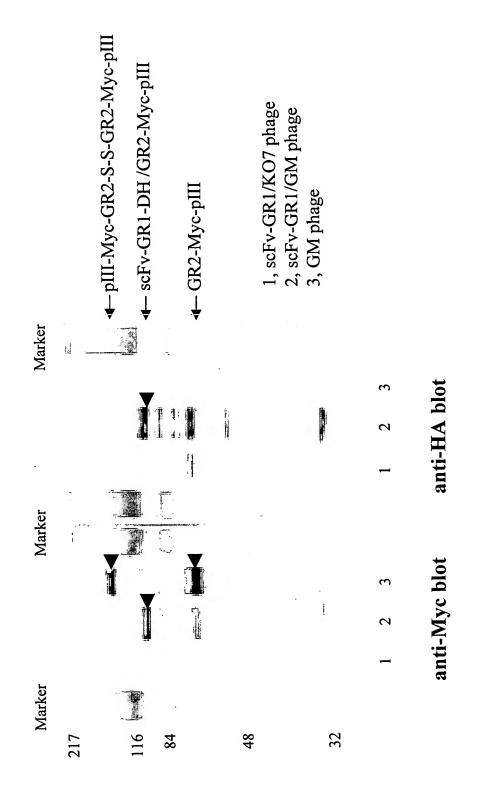
Complete vector sequence of pABMX14

GCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATGGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGC CTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATTATCTAACACGACGGGGAGTCAGGCAACTA IGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATAÏATACTTTTAGATTGATTTAAAAACTTCATTTTAAATAAAGG TCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTTTACCGGG AGAAAGCGCCACGCTTCCCGAAGGGAGAAAAGGCGGACAGGTATCCGGTAAGCGGCTCGGAACAGGAGGAGGAGGAGGAGGTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCG ATGITCITICCTGCGITAICCCCTGAITCTGTGGAIAACCGIAITACCGCCTITIGAGTGAGCTGAIACCGCTCGCCGCAGCCGAACGACGAGCGCAGCGAGTCAGTGAGCGAAGCGAAGAAGA GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACCGGTTCTTTAAGGAGGA TTGGAGAAGGAGAACCGTGAACTGGAAAAGATCATTGCTGAGAAAAAAGAGGGGGGTGTCTCTGAACTGCGCCATCAACTCTGTAGGAGGTTGTAGATCTTATCCATACGACGTACCAGACTA CGCAGGAGGTCATCATCATCATCACCATTAATGAGTCGACCTCGACCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGAACTGGGAAAACCCTGGCGTT ACCAACTTAATGGCCTTGCAGCACCCTTTGGCCAGCTGGCGTAATAGGGAAGAGGCCGGATCGCCTTCCCAACAGTGGCGCAGCCTGAATGGCGAATGGGACGCGCCTGTAG CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTTAAAGGGATTTTGCCGATTTCGGCCTATTG gttaaaaatgagctgatttaacaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttc3gggaaatgtgcgcggaacccctatttgtttatttttttaaaa CCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGGGGGGTATTATCCCGTATTGACGCGGGGCAAGÄGCAACTCGGTCGGCGGATACACTATTCTCAGAATGACTTGGT GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGTTTTT GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

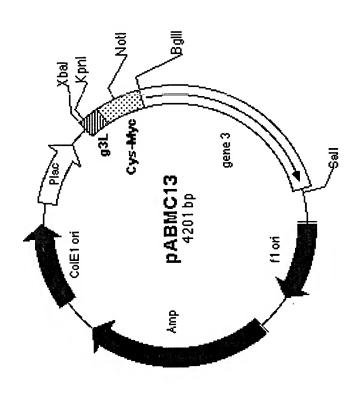
Functional display of scFv by GM-UltraHelper phage



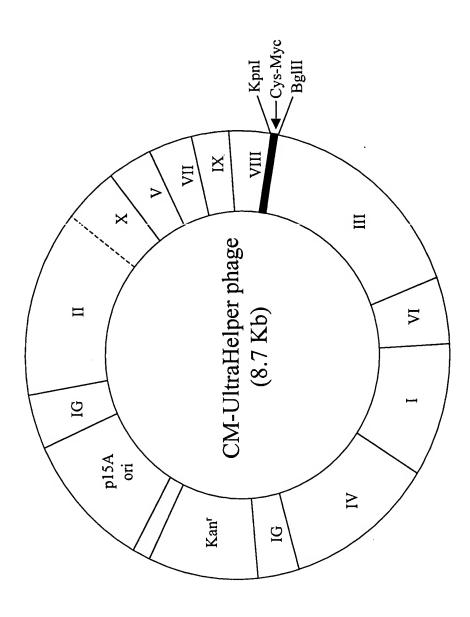
Mutivalent display of scFv by GM-UltraHelper phage



Map of phagemid vector pABMC13



Helper phage with Cys-Myc-pIII fusion gene

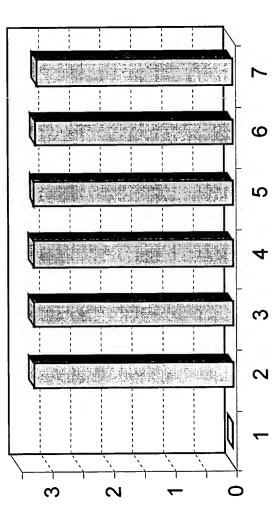


Engineered gene III sequence in CM phage

---TTAGT<u>GGTACC</u>TTTCTATTCTCACTCCGCT <u>TAG</u>GCTTGCGGTGGT<u>GCGGCCGC</u>AGAACAAAAACTCATCTCAGAAGAAGAGGATCTGAGATCT <u>AGATCT</u>GGA Myc-tag O K L I S E GAAAE NotI ט KpnI Gene III leader Amber stop S H S A * A C

GGCGGT ACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCT-----W K D D K > N H Œ ഗ z 臼 H Ħ Д × r A υ യ Gene III T VE

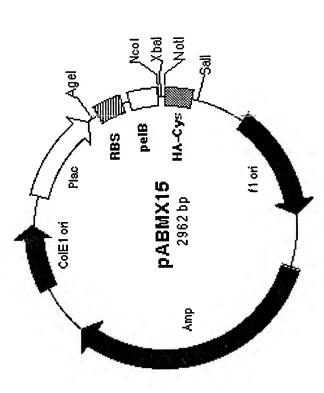
Detection of Myc-tag on CM-UltraHelper phages by ELISA



Phage binding to anti-Myc antibody (OD405)

KO7 phage
CM phage clone A9
CM phage clone B6
CM phage clone B7
CM phage clone B8
CM phage clone B8
CM phage clone B9
CM phage clone B9

Phagemid vector for protein-HA-cys expression

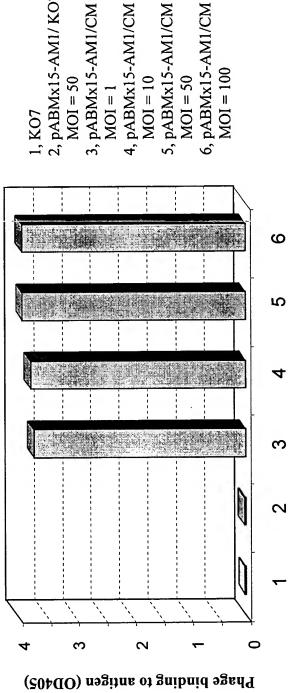


rockson allokol

Complete vector sequence of pABMX15

GCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAA GGTGGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCTGAGTTAGGCCACCACCACCACAAAAAAACTCTGTAGTTGCCGA CHACTACTICGCTCTGCTAAICCTGTTACCAGTGGCTGCTGCCGGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGGGGGTCGGGCTGAACGGGGGGTTCGT A PARABABATGABA TACCTATTGCCTACGCGGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGAGGCGCCGCTTACCCGTACGACGTTCCGGACTACGCAGGTGGCT alticcentiticcentalticcettititiecgeaittitieccticctettitieccticcagaacectegtgaaagtaaaagatecteaagatcagteggtecacgagtegettacatcgaactegatetca **3CGCAACGCAATTAATGTGTGGTTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGGAATTGTGAGCGGATAACAATTTACCTGTTCTTTTAACTTAAGGA** GCTGATAAGTCGACCTCGACCTATTAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGAGTAAACCCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACTTCCCCCTTTC CTCGAACACCCAAAAAACTIGATTAGGGTGATGGTTCACGTGGTGGGGCCATCGGCCGTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC aaacctaicticggtctaitcititgaitiataagggaittitgccgaitticggcctaitggcstaaaaaatgagctgaittaacaaaaattitaacaaaaaaataitaa COGIAAGAICCTIGAGAGITITICGCCCCGAAGAACGITITICCAAIGAIGAGCACTITITAAAGITICTGCTAIGIGGCGGGTATIATICACGCTGGGCGGGGAAGAGCAACTCGGTCGCCGGATAGACA

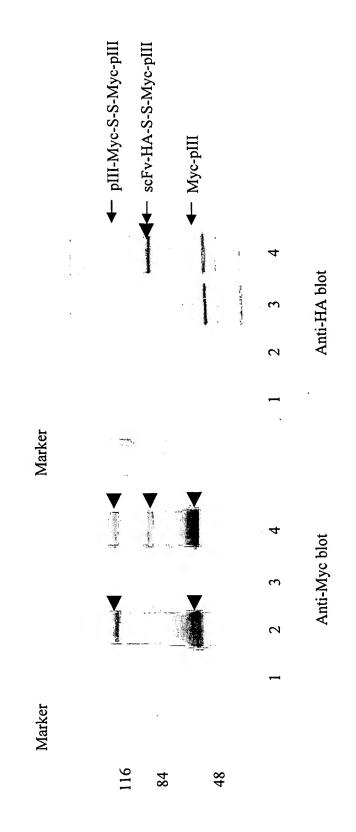
Functional display of scFv by CM-UltraHelper phage



4, pABMx15-AM1/CM MOI = 10 5, pABMx15-AM1/CM MOI = 50 1, KO7 2, pABMx15-AM1/ KO7 3, pABMx15-AM1/CM MOI = 1

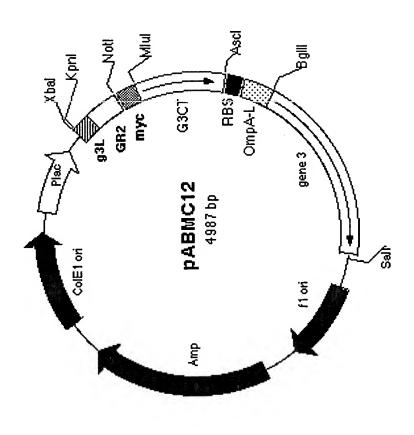
MOI = 100

Detection of scFv displayed by CM-UltraHelper phage

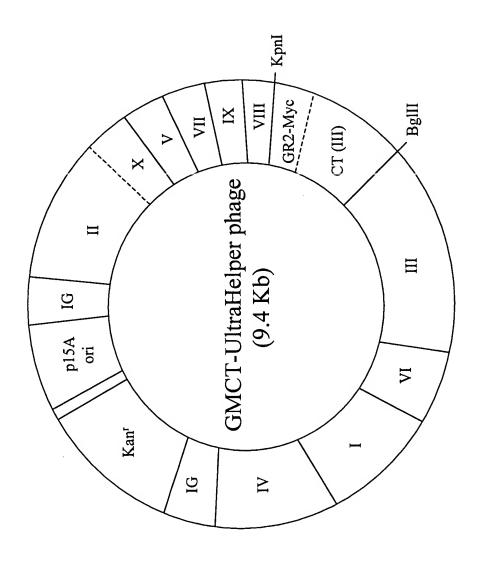


1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/KO7; 4: pABMx15-AM1/CM

Map of phagemid vector pABMC12



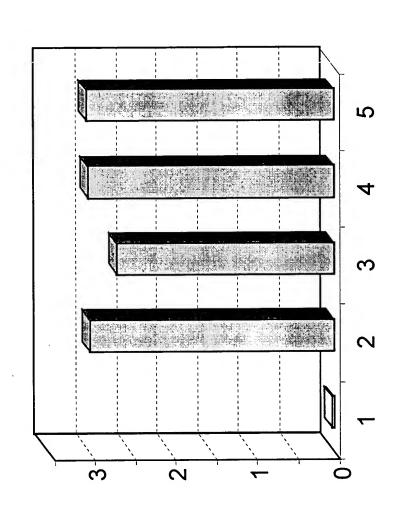
Helper phage with an additional copy of engineered gene III



Emgineered gene III Sequence in GMCT phage genome

--TTAGTGGTACCTTTCTATTCTCACTCCCGCT ACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAA CTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGC GGCTCTGGTGGTGGTTCTGGTGGCGGCTCTGAGGGTGGCGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTTGCGGTGGCGGCTTCC GGTTCCGGTGATTTTGATTATGAAAAATGGCAAACGCTAATAAGGGGGCTTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAAGGCAAAA TITATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAATAA GGCGCCCACAATTTCACAGTAAGGAGGTTTAATAA ATGAAA GACTTGGAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC GCGGCCGCAGAACAAAACTGATCTCAGAAGAGGATCTGACGCGTGCT GGCGGC AAGACAGCTATTGCGATTGCAGTGGCACTGGCTTTCGCTACCGTAGCGCAGGCT <u>AGATCT</u>GGAGGCGGT ACTGTTGAAAGTTGTTAGCAAAA---× Ω A T Gene III D L œ щ Ö × ט ø Σ ט ENAL S L 团 田 G L A N G N G V F ENHRLR 臼 Myc-tag в о у г р တ ഗ Ö GR2 domain O K L I ט œ MTENAD ט ט ŋ CDKINLF AscI BglII ഗ ഠ æ တ SPLMNNF ഗ ഗ A A A E ŋ D Q Ø ט Ø r L NotI უ K G A ø യ 臼 H H > EЗ 闰 U ഗ O I S × H Ы ტ ט ŋ z œ FDYEKMANAN ď υ ß ט C D A T D Y G A A I D ĸ ſΞĄ Ö Ы > ſΞų ט H ы о О 臼 н ø Gene III Leader ഗ ο ο ο K P Y z ы S ø ט Н Ø Ħ > ט Ø ĹŦ CT domain of Gene III හ Н ď U T F. M ø ഗ н ט ď ტ [z, KpnI ը > > Ω FMYV OmpA leader

Functional display of scFv by GMCT-UltraHelper phage



- 1, pABMx14-AM1/ KO7 MOI = 50
- 2, pABMx14-AM1/GMCT MOI = 1
 - 3, pABMx14-AM1/GMCT MOI = 10
- 4, pABMx15-AM1/GMCT MOI = 50
 - 5, pABMx14-AM1/GMCT MOI = 100

Detection of scFv displayed by GMCT-UltraHelper phage

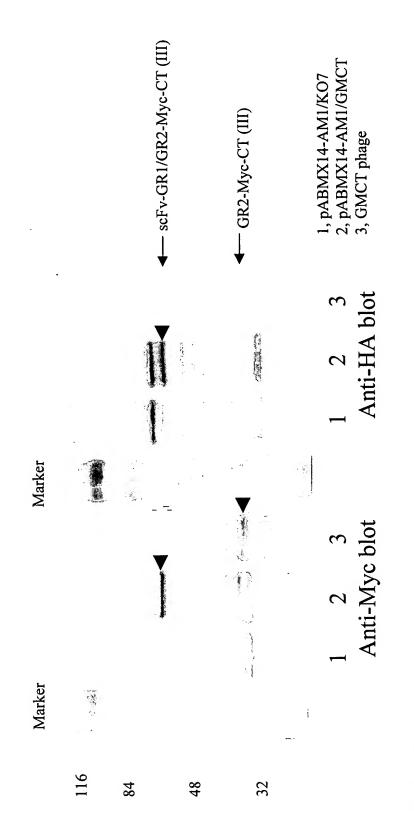
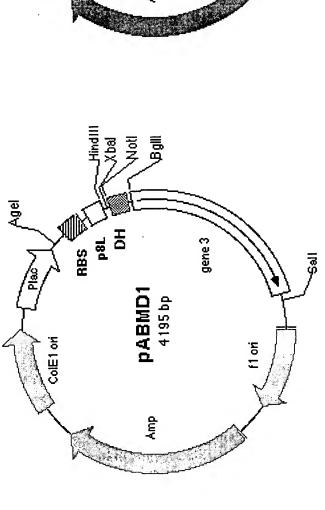


Fig. 21



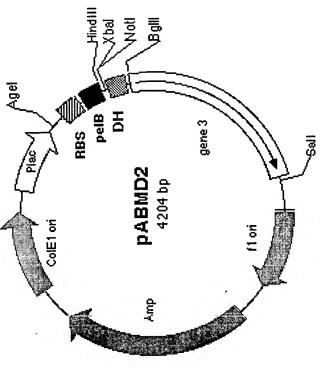


Fig. 22A

PABMID1 vector: sequence from Agel to Sall

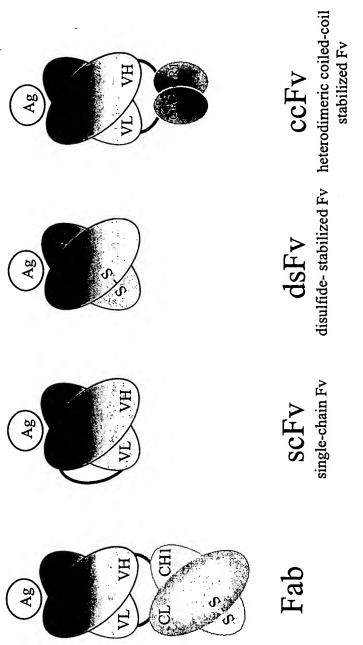
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA <u>GTCGAC</u> AGATCT ATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCT<u>AAGCTT</u>CGCT Amber stop BgIII Sall S R SVAVATLVPMLSFA GCGCCCCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG HindIII AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA Y P Y D V P D Y A G G H H H H H H His-tag A N Gene 3 SLVLKA HA-tag ഗ 团 P8 Leader lac promoter/lac O1

PABMID2 vector: sequence from Agel to Sall

<u> ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTTCTAGA</u> LIPTAAAGLLILAAQPAMAALQA GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC AGATCT Amber stop BglII GCGCCCCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA Nco I His-tag S/D C L A K ---- A N I L <u>ი</u> $\mathbf{E}\mathbf{b}$ Y P Y D V P D Gene 3 Agel HA-tag മ pelB Leader 臼 lac promoter/lac O1

GR1 Sequence Range: 1 to 146

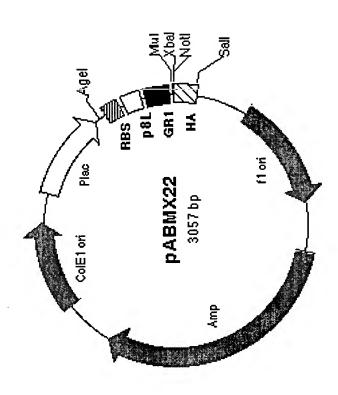
GR2 Sequence Range: 1 to 140



antigen Ag

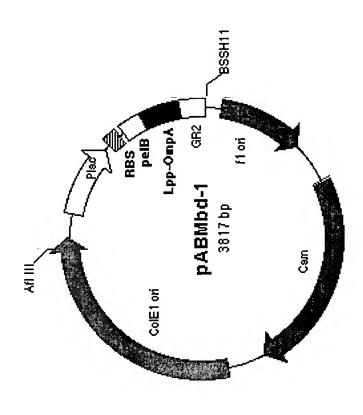
Fig. 24

Expression vector for Adapter-directed bacterial display



Complete vector sequence of pABMX22

aaggaagagtatgagtattccaacatttccgtgtcgcccttattcccttttttgcggcattttgcccttcctgtttttgctcagaaaggtaaaagatgctgaagatgctga TACTITAGATTGATTTAAAACTTCATTTTAAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAA;CCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA acaacactcaaccctatctctattttattataagggattttgccgattttcgcccatttggcctattggcctattggcctattgacaaaattaaccaaaatttaacgcgaattttaacaaaaatataacgcc CACGAGTGGGTTACATCGAACTGGATCTCAACAGGGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAAGTTTAAAAGTTCTGCTATGTGGCGGGGGTATTATCCCGTATT gacgccggcaagagcaactacccgcatacactattctcagaatgacttggttgactcaccagaaaaaag&atcttacggatggcatgacagtaagagaattatgcagtgctgccat CTCCCGTATCGTAGTTATCTACACGGGGGGGGGGGGGCGACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATAA aactggcttcagcagaggggaatactgtccttctagtgtagccgtagttaggccaccaccacttcaagaactctgtagcaccgcctacatacctcgctcataatcctgttaccagtggctg CTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGGGGGTTCGTGCACACCAGCCCAGCTTGGAGCGAACGACCTACACC gaactgagatacctacagcggggctatgagaagcgccacgcttcccgaaggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaa CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCCAGCAACGCGGCCTTTTTACGGTTCCTGG aaaaaatgaaaaagtetttagtecteaaageeteegtagegstacetegtteegatgetaagettegetggtgaggaaaagteeggtggagaaaagagaacegtgaactggaaaaga attgctgagaagaggaggtttctgaactgcgccatcaactgcagtctgtaggcggttgcacgcgttctagagcggcc;cttacccgtacgacgttccggactacgcatgataagtcgacctcga **CCAATITCGCCCTATAGTGAGTGGTTTTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTA**CCC**TAATCGCCTTGCAGCACTTACGCCGTTT**CGCCGTTTGGCCGTTTCGCCGAGCTTACGCCGT aatagcgaaggcccgcaccgatcgcccttcccaacagtgcgcagcctgaatggcgatggcgacgccctgtagcggc;cattaagcggcgggtggtggtggtggtggcgcgg **CCTCGAADAAA**CTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA\GTTGGAGTCCACGTTCTTTAATAATAGTGGACTCTTGTTGCAAACTGGA aaccatgagtgataacactgcggccaacttacttctgacaaccgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcGttgggaaccggagctga Helper vector for adapter-directed bacterial display



loossee lacal

Complete vector sequence of pABMXbd-1

TGATTACGCCAAGCGCGTTTAACTTTAGTAAGGAGGAATTAAAAATGAAATACCTGCTGCCGACCGCAGCCGCGGGTTTGCTGTTACTGGCGGCCCAGCCGGCTATGGCGATGAAAGCTACTAAAACTG GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGGGGATAACAATTTCACACAGGAAACAGCTATGACCA gtactgggcaacccgtatgttggctttgaaatgggttacgactggttaggtcgtatgccgtacaaaggcagcgttgaaaacggtgcatacaaagctcagggcgttcaactgaccgctaaactgggttac CCAATCACTGACGACCTGGACATCTACACTCGTCTGGGTGGCATGGTATGGCGTGCAGACACTAAATCCAACGTTTATGGTAAAACCACGACACCGGCGTTTCTCCGGTGTTTCGCTGGCGGTGTTGAG IACGCGATCACTCCTGAAATCGCTACCCGTCTGGAATACCAGTGGACGAACAACATCGGTGACGCACACACCATCGGCACTGGACGGAGGGTACATCCCGGCTGGAGGGCCTACAGTCAGAAAAC CATCGCCTGCGAATGAAGATCACAGAGGTGGATAAAGACTTGGAAGAAGTCACCATGCAGCTGCAAGACGTTGGCGGTTGGTÄATGAGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA aaccetggcgttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaaggcccgcaccgätcgccttcccaacagttgcgcagcctgaatggcgaatgggacgcg CGCCCTITGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTGGTCTÄTTCTTTTGATTTAAAGGGATTTTGCCGATTTCGGCCTATTGGTTA TGAGAATATGTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTC?:TCGCCCCGTTTTCACCATGGGCAAATATATACGCAAGGCGACAAG atatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccgtattttgcgt ITGCTCACCCAGAAACGCTGGTGAAAGTAAAABATGCTGAAGATCAGTTGGGTGCCACGAGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCCGAAGAACGTTTTC caagititaicoggectitaticacatictigecegectgaigaaigcicatecggaattacgtaiggcaatgaaagacggtgagetggtatgggatagtgttececettgttacaecgittieca TGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATTTCGCAAGATGTGGCGTGTAACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTAT CCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTÄGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTT TCTATTGCTGGTTTACCGGTTTATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCCTGAGGCCAGTTTGCTCA¢GCTCTCCCGTGGAGGTAATAATTGACGATATGATCCTTTTTTTCT gatcaaaaaggatctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgttttcgttccactgagcktcagaccccgtagaaagatcaaaggatcttgagatctttt CTGTCCTTCTAGTGTAGCGGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTAATCCTGTTAACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGT tggactcaagacgatagttaccggataaggcgcagcggttggagcgggggttcgtgcacacagcctggcagcgáacgagcgaactacaccgaactgagatacctacagcgtgagctatgagaaa CGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCCGACTGGAAAGCGGGCAGTGA